Results: Cartilage cultured in the presence of blood showed a decrease of proteoglycan synthesis rate of 70%, an increase of proteoglycan release of 100%, and a decrease of proteoglycan content of 15% after 16 days of culture (all $p<0.05$). This blood-induced damage of the cartilage matrix was limited by IL-4 in a clear dose-dependent way. Addition of 100 ng/ml IL-4 during blood-exposure reduced the proteoglycan synthesis rate with only 45%, and decreased the proteoglycan release with 30% compared to control (all $p<0.05$). Moreover, proteoglycan content was normalized. The combination of IL-4 and IL-10 was clearly more protective against damage caused by blood. This was especially evident for the proteoglycan synthesis which was completely normalized. Furthermore, treatment with a combination of the two cytokines was significantly better than the effect of IL-4 and IL-10 alone ($p<0.05$).
Conclusions: Besides IL-10, as shown previously, also IL-4 protects against blood-induced cartilage damage. The combination of these two cytokines is clearly the most protective. In addition to the direct effects on cartilage, both cytokines are synergistic in inhibition of inflammation. As such, this justifies further evaluation of the combination of IL-4 and IL-10 in prevention and treatment of blood-induced joint damage.

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BRADYKININ, THROUGH B_2 RECEPTORS, ACTIVATES THE RELEASE OF THE CYTOKINE INTERLEUKIN 6, THE CHEMOKINE INTERLEUKIN 8, AND THE METALLOPROTEINASE 3 IN HUMAN KNEE CHONDROCYTES

S. Meini, P. Cucchi, F. Bellucci, C. Catalani, S. Giuliani, C.A. Maggi
Menarini Ricerche S.p.A., Florence, Italy

Purpose: Bradykinin (BK, H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) is a proinflammatory and algogenic peptide: it releases inflammatory mediators and sensitizes sensory afferents through the activation of B_2 receptors expressed on the membrane of several cell types, including synoviocytes and chondrocytes. Aim of the current investigation was to investigate if BK and B_2 receptor can participate into mechanisms which are involved into ostearthritis (OA) degenerative events, and to evaluate the possibility to prevent them through B_2 receptor blockade, using the highly selective and potent B_2 receptor antagonist MEN16132 (m.w. 873.16).
Methods: Human chondrocytes (Lonza, CC-2550) were cultured in F12/DMEM 1:1 added with CGM Singlequots (Lonza, CC-4409) and Gln 2mM and used up to fifth passage. Experiments were performed with cells at confluence plated onto 24-well plates. Cells were incubated at the indicated concentration of BK in F12 medium supplemented with foetal bovine serum (1%), penicillin (50 μg/ml), streptomycin (50 μg/ml), amphotericin B (0.75 μg/ml), Gln (2 mM), and captopril (1 μM). At the end of the experiments, surnatants were collected and stored at -80°C and used for the dosage of interleukin 6 (IL-6), interleukin 8 (IL8), and metalloproteinase 3 (MMP3). IL-6, IL-8, and MMP3 contents in the supernatant were assayed by commercially available enzyme immunoassay kits (Promokine PK-EL-61606, PK-EL-61806, and Biosource KAC1541). Data are expressed as mean ± s.e.m. or 95% confidence limits in parentheses of 3 to 5 experiments, each in triplicate.
Results: Time-course experiments (2 - 96 h) indicated that BK (1 μM) induced a release of IL-6, IL-8, and MMP3 which increased over the time, stably peaked after 24 h of incubation, and remained constant up to 96 h. The maximal production of IL-6, IL-8 and MMP3 induced by BK (24 h incubation) was 557±39 pg/ml, 1267±169 pg/ml, and 5.52±0.73 ng/ml, respectively and was resembling that induced by the pleiotropyc cytokine TNFα (0.1 ng/ml)(817±350 pg/ml, 1778±198 pg/ml, and 1.96±0.2 ng/ml, respectively). Concentration-response curves to BK (0.1 nM - 1 μM, 24 h) indicated EC_{50} values of 10 nM (5.4-18.6) in inducing an augmented release of IL-6, 9.7 nM (3.7-32.5) for the release of IL-8, and 9.1 nM (1.1-7.6) for MMP3 secretion. This effect provoked by BK (100 nM, submaximal concentration) were concentration-dependently prevented by the pretreatment of HCCK (30 min) with the selective B_2 receptor antagonist MEN16132, and IC_{50} values were 1.7 nM (1.0-2.8) to inhibit IL-6 production, 2.2 nM (0.7-7.1) for IL-8, and 0.7 nM (0.3-1.4) for inhibition of MMP3 secretion.
Conclusions: These findings disclose novel actions of BK that imply its possible involvement in joint degenerative diseases, and indicate B_2 receptor blockade as a potential therapy in OA pathology.

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CARTILAGE THICKENING IN EARLY RADIOGRAPHIC KNEE OSTEOARTHRITIS - FACT OR ARTIFACT?

S. Cotofana[1], W. Wirth[1], R.J. Buck[2], F. Roemer[3], D. Dreher[4], M. Nevitt[5], F. Eckstein[1]
[1]*PMU-Salzburg, Salzburg, Austria;* [2]*StatAnswers Consulting LLC, San Diego, CA;* [3]*Quantitative Imaging Ctr., Dept. of Radiology, Boston, MA;* [4]*Merck Serono, Geneva, Switzerland;* [5]*UCSF, San Francisco, CA*

Purpose: In animal studies of osteoarthritis (OA), increases in cartilage thickness have been observed at the early phases, due either to cartilage hypertrophy or edema. Evidence for cartilage thickening in human OA, however, remains enigmatic. Cross sectional analyses and one longitudinal study in human with MRI have indicated that medial osteophytes, in the absence of medial joint space narrowing (JSN), may be associated with cartilage thickening in the medial femorotibial compartment in human OA, particularly in the external aspect of the medial femoral condyle (ecMF). However, comparisons may have been confounded by differences in age, BMI and other factors.
To eliminate confounding by between-person differences and to enhance sensitivity, we performed a within-person between-knee analysis of cartilage thickness in knees with unilateral osteophytes and without JSN. The primary endpoint was the between-knee cartilage thickness differences in ecMF (in subjects with unilateral medial osteophytes), the secondary endpoint was thickness differences in the external lateral femur (in those with unilateral lateral osteophytes), and the exploratory endpoints were thickness differences in all other femorotibial subregions.
Methods: 83 OA Initiative participants were selected from 4800 cases (public use data sets 0.E.1 [imaging] and 0.2.2 [clinical]), who displayed definite osteophytes and no JSN in one knee and no signs of radiographic OA in the other knee, according to the site readings. The radiographs were reviewed by a central reader (F.R.) and 61 participants were included. Cartilage thickness was measured in four femorotibial cartilage plates (medial/lateral, femur/tibia) and in 16 femorotibial subregions, using sagittal DESSwe MR images and dedicated software (Chondrometrics). Within-person between-knee differences were calculated with a paired t-test and general linear models. No adjustment for multiple comparisons was performed.
Results: Of the 61 participants (age 60.8±9.6 yrs; 29m/32f; BMI 27.8±4.7) 48% displayed medial, 36% lateral, and 16% bicompartimental (mostly tibial) osteophytes. Knees with osteophytes had thicker cartilage than contralateral knees in the external medial (ecMF:+5.5%,p=0.02) and external lateral femur (ecLF:+4.1%,p=0.01), but in no other subregions. Knees with only medial osteophytes displayed greater thickening in ecMF (+7.8%) than ecLF (-0.1%), those with only lateral osteophytes greater thickening in ecLF (+5.0%) than in ecMF (+1.7%), and those with bicompartimental osteophytes similar thickening in both subregions (+17%/+15%, respectively). There was no significant effect of age, sex, or BMI on between-knee differences.
Conclusions: Knees with early radiographic OA (definite osteophytes, no JSN) displayed thicker cartilage than the contralateral knees without radiographic OA. The differences were specific to external medial femoral and external lateral femoral subregions and appeared to be locally mediated by (tibial) osteophytes in the same compartment.

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SUPEROXIDE DISMUTASE 2 DOWNREGULATION AND MITOCHONDRIA RESPIRATION IN OSTEOARTHRITIS

C. Gabrielides[1], J.L. Scott[1], R.W. Taylor[2], D.A. Young[1]
[1]*Musculoskeletal Res. Group, Inst. of Cellular Med., Newcastle Univ., Newcastle Upon Tyne, United Kingdom;* [2]*Inst. for Ageing and Hlth., Newcastle Univ., Newcastle Upon Tyne, United Kingdom*

Purpose: Oxidative phosphorylation takes place at the mitochondrial respiratory chain and is the major source for the production of ATP. A by-product of this respiration are reactive oxygen species (ROS). ROS are involved in signalling processes but when at high levels contribute to oxidative damage. The major ROS include superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). The mRNA and protein levels of the major inhibitor of superoxides in the mitochondria, superoxide dismutase 2 (SOD2), have been shown to be downregulated in osteoarthritic compared to healthy joint cartilage.
This study characterises the effects of reduced levels of SOD2 on chondro-